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Dietary lipid levels affect lipoprotein clearance, fatty acid transport, lipogenesis and lipolysis at the transcriptional level in muscle and adipose tissue of large yellow croaker (*Larimichthys crocea*)

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Abstract

To investigate the effect of dietary lipid levels on lipid metabolism in muscle and adipose tissue of large yellow croaker (*Larimichthys crocea*), fish (average body weight 150.0 g) were fed to one of three diets with a low (6%), moderate (12%, the control diet) or high (18%) crude lipid level for 10 weeks. The expression of genes related to lipoprotein clearance (*lpl*, *ldlr*, *lrp1* and *srbi*), fatty acid transport (*cd36*, *fatp1*, *fabp3* and *fabp11*), lipogenesis (*fas* and *dgat2*) and lipolysis (*atgl*, *cpt1* and *aco*) in muscle and adipose tissue was analysed. In muscle of fish fed the low-lipid diet, the expression of *lpl*, *lrp1*, *fas*, *dgat2* and *atgl* was significantly downregulated, whereas *fabp3* and *fabp11* were significantly upregulated compared with those fed the control diet. In muscle of fish fed the high-lipid diet, the expression of *lpl*, *ldlr*, *lrp1*, *cd36*, *fabp3*, *fas* and *dgat2* was significantly decreased, while *fabp11* expression was significantly increased compared with those fed the control diet. Compared with the control group, only a significant downregulation of *fabp3* expression in adipose tissue was observed in the low-lipid group. In the high-lipid group compared with the control group, the expression of *ldlr*, *fabp11*, *dgat2*, *atgl* and *aco* in adipose tissue was significantly upregulated, whereas *cpt1* expression in adipose tissue was significantly downregulated. These results indicated that the expression of the selected key genes related to lipid metabolism was tissue-specific regulation by dietary lipid levels in large yellow croaker.

Keywords: dietary lipid levels, fish oil, lipid deposition, lipid transport, lipoprotein receptor, fatty acid uptake, large yellow croaker

Introduction

Driven by the protein-sparing effect of lipids, high-lipid diets are commonly used in aquaculture (Todorčević, Vegusdal, Gjøen, Sundvold, Torstensen, Kjær & Ruyter 2008). However, high-lipid diets also increase lipid deposition in fish, and compromise fish health and product quality (Du, Clouet, Zheng, Degrace, Tian & Liu 2006; Lu, Xu, Wang, Zhang, Zhang & Liu 2014). Thus, dietary lipid level and its metabolic effects must be evaluated very carefully to prevent excessive lipid deposition in cultivated fish. Understanding of metabolic effects of dietary lipid levels on fish lipid metabolism may be necessary in developing strategies to prevent lipid deposition.

To date, many studies have been conducted to investigate the effects of dietary lipid levels on lipid metabolism in the liver of fish (Wang, Liu, Tian, Mai, Du, Wang & Yang 2005; Du *et al.* 2006; Antonopoulou, Kousidou, Tserga, Feidantsis & Chatzifotis 2014; Lu *et al.* 2014; Wang, Li, Hou, Gao & Wang 2015). As tissues with high rates of lipid metabolism, however, the effects of dietary lipid levels on lipid metabolism in muscle and adipose tissue have been little investigated in fish (Jobling, Koskela & Savolainen 1998; Hemre & Sandnes 1999; Nanton, Lall, Ross & Mcniven 2003). Like liver, lipid uptake in muscle and adipose tissue is mediated by lipoprotein lipase (LPL),

cluster of differentiation (CD36), a family of fatty acid transport proteins (FATP1-6), plasma membrane-associated fatty acid-binding proteins (FABPpm), LDL receptor (LDLR), LDL receptor-related protein-1 (LRP1) and scavenger receptor class B type I (SRBI) (Descamps, Bilheimer & Herz 1993; Acton, Rigotti, Landschulz, Xu, Hobbs & Krieger 1996; Goldberg, Eckel & Abumrad 2009; Glatz, Luiken & Bonen 2010). Once transported into adipocytes and myocytes, lipids are targeted to catabolic and anabolic pathways through fatty acid-binding proteins (FABPs) (Storch & Thumser 2010). In addition, adipose triglyceride lipase (ATGL) (Arner & Langin 2014), carnitine palmitoyltransferase I (CPTI) and acyl-CoA oxidase (ACO) are key enzymes in lipid catabolic metabolism (Lopaschuk, Ussher, Folmes, Jaswal & Stanley 2010), while fatty acid synthase (FAS) and acyl-CoA: diacylglycerol acyltransferase 2 (DGAT2) play important roles in lipogenesis (Coleman & Lee 2004). However, some differences in lipid metabolism are observed among liver, muscle and adipose tissue due to various metabolic roles. In fish, *de novo* lipogenesis mainly takes place in the liver and is negligible in muscle and adipose tissue (Wang *et al.* 2005). Furthermore, unlike liver, lipoprotein secretion does not occur in muscle and adipose tissue (Goldberg *et al.* 2009). These data seem to indicate that the effect of dietary lipid levels on lipid metabolism in muscle and adipose tissue is different from that in the liver.

In fish, it was reported that dietary lipid levels could affect *lpl* expression, lipid catabolic metabolism and lipogenesis (Han, Wen, Zheng & Li 2011; Lu, Xu, Li, Liu, Wang & Zhang 2013; Yan, Liao, Wang, Mai, Xu & Ai 2015). In addition, in rainbow trout, *cd36* and *fatp1* mRNA levels in adipose tissue (Torstensen, Nanton, Olsvik, Sundvold & Stubhaug 2009), and *ldlr* mRNA level in the liver and adipose tissue (Richard, Kaushik, Larroquet, Panserat & Corraze 2006) were changed with vegetable oils replacement of fish oil. Furthermore, *fabps* mRNA levels were regulated by dietary fatty acids in Atlantic salmon and zebrafish (Jordal, Hordvik, Pelsers, Bernlohr & Torstensen 2006; Karanth, Lall, Denovan-Wright & Wright 2009; Venkatachalam, Sawler & Wright 2013). Moreover, we previously showed that dietary lipid levels regulated the expression of *ldlr*, *lrp1*, *cd36*, *fatp1*, *fabp11* and *fabp3* in the liver of large yellow croaker (Yan *et al.* 2015). Collectively, these data strongly support the hypothesis that lipid metabolism in muscle and adipose tissue may also be influenced at the transcriptional level by dietary lipid levels in fish. However, there has been no study to confirm this hypothesis.

Large yellow croaker (*Larimichthys crocea*) is widely studied in China due to its high production and critical role in human food. In our previous study, we found that dietary lipid levels regulated lipid transport, uptake, synthesis and catabolism in the liver of large yellow croaker (Yan *et al.* 2015). Here, to further clarify how dietary lipid levels influence lipid metabolism, we examined the effects of dietary lipid levels on lipid uptake, lipid catabolic metabolism and lipogenesis at the transcriptional level in muscle and adipose tissue of large yellow croaker.

Materials and methods

Ethics statement

This study was performed according to the Standard Operation Procedures (SOPs) of the Guide for the Use of Experimental Animals of Ocean University of China. All animal care and use procedures were approved by the Institutional Animal Care and Use Committee of Ocean University of China (Permit Number: 20001001).

Experimental diets and fish

Three isoproteic (crude protein 43%) diets were formulated to contain graded levels of lipid (6, 12 and 18% on a dry basis) (Table 1, referred to Yan *et al.* (2015). The diet with 12% crude lipid was used as the control because this dietary lipid level has been proved to be optimal for the growth of large yellow croaker (Yi, Zhang, Xu, Li, Zhang & Mai 2014). All ingredients were ground into fine powder so that they passed through a 246-μm screen. Ingredients of each diet were blended thoroughly first by hand and then mechanically. The fish oil was then thoroughly mixed with all ingredients of each diet, after which water (200 g kg^{-1}) was added to make stiff dough. Pellets were made using an automatic pellet-producing machine (Weihai) and dried for about 12 h in a ventilated oven at 40°C. After drying, the diets were stored at −20°C during the experiment.

The study was conducted in a local farm in Xihu Bay, Ningbo, China. Fish were reared for 2 weeks to acclimate the experimental conditions.

Table 1 Formulation and proximate composition of the experimental diets

	Dietary lipid levels (%)		
	Low (6)	Moderate (12)	High (18)
Ingredients (g 100 g^{-1})			
Fish meal*	39	39	39
Soybean meal*	20	20	20
Wheat meal*	23.3	23.3	23.3
Wheat starch*	12	6	0
Fish oil*	0	6	12
Soybean lecithin*	1.5	1.5	1.5
Vitamin premix†	2	2	2
Mineral premix‡	2	2	2
Attractant§	0.1	0.1	0.1
Mould inhibitor¶	0.1	0.1	0.1
Proximate composition (g 100 g^{-1})			
Moisture	9.5	9.4	9.2
Crude protein	43.1	42.6	43.2
Crude lipid	6.1	11.5	17.8

*All of these ingredients were supplied by Great Seven Biotechnology, China.

†Vitamin premix (mg or g kg^{-1} diet): cholecalciferol, 5 mg; retinol acetate, 32 mg; thiamin 25 mg; vitamin B_{12} (1%), 10 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; ascorbic acid, 2000 mg; alpha-tocopherol (50%), 240 mg; vitamin K_3, 10 mg; pantothenic acid, 60 mg; inositol, 800 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin (2%), 60 mg; choline chloride (50%), 4000 mg; microcrystalline cellulose, 12.47 g.

‡Mineral premix (mg or g kg^{-1} diet): $CuSO_4 \cdot 5H_2O$, 10 mg; $Ca(IO_3)_2 \cdot 6H_2O$ (1%), 60 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $FeSO_4 \cdot H_2O$, 80 mg; $MgSO_4 \cdot 7H_2O$, 1200 mg; $MnSO_4 \cdot H_2O$, 45 mg; $NaSeSO_3 \cdot 5H_2O$ (1%), 20 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $CaH_2PO_4 \cdot H_2O$, 10 g; zeolite, 8.485 g.

§Attractants: glycine and betaine.

¶Mould inhibitor: contained 50% calcium propionic acid and 50% fumaric acid.

At the start of the experiment, fish were fasted for 24 h. After anaesthetized with eugenol (1:10 000) (Shanghai Reagent, Shanghai, China), fish (average body weight 150.0 g) were randomly distributed into nine cages (1.5 × 1.5 × 2.0 m) with 40 fish each cage. Each diet was randomly allocated to triplicate cages, and fish were hand-fed to apparent satiation twice daily for 10 weeks under natural light conditions. Water temperature, salinity and dissolved oxygen were measured and recorded as 21–28.5°C, 28–32‰ and 6.7–7.8 mg L^{-1} respectively. At the end of the experiment, fish were fasted for 24 h and anaesthetized with eugenol (1:10 000) (Shanghai Reagent). Then, the dorsal muscle and abdominal adipose tissue of four fish in each cage were collected into plastic bags and then stored at −20°C for the assay of moisture and crude lipids. The left-side dorsal muscle (a mix of fast and slow muscle) in the middle of lateral line and dorsal fin, ranged from the origin of dorsal fin to approximately a quarter of dorsal fin, and abdominal adipose tissue of three fish in each cage were pooled into 1.5-mL tubes (RNase-Free; Axygen), frozen in liquid nitrogen and then stored at −80°C for later analysis of gene expression.

Proximate composition and fatty acid analysis

The moisture content of the muscle and adipose tissue was determined using the freeze-drying method (Christ ALPHA, Osterode am Harz, Germany). The crude lipid content of these two tissues was measured according to the Folch, Lees and Sloane-Stanley (1957) method and quantified gravimetrically.

RNA isolation and real-time quantitative PCR

Total RNA was isolated from muscle and adipose tissue using the standard TRIzol (Invitrogen, Carlsbad, CA, USA) extraction method and dissolved in diethylpyrocarbonate (DEPC)-treated water. The quality of RNA was analysed on a 1.2% denaturing agarose gel, and the total RNA concentrations were measured using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Thereafter, 1 μg of RNA was reverse-transcribed using a Transcript One-Step gDNA removal and cDNA Synthesis SuperMix kit (Transgen Biotech, Beijing, China) following the manufacturer's protocol.

Primers (Table 2) for the real-time quantitative PCR were designed using the Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, Canada). Primers were synthesized and the qPCR products of each gene were confirmed through sequencing by Shanghai Boshang Biological Technology, China. Real-time PCR was performed with the Eppendorf Mastercycler ep realplex (Eppendorf AG, Hamburg, Germany) using a Top Green qPCR SuperMix kit (Transgen Biotech, Beijing, China). A 10 μL of 2 × TransStart Top green qPCR SuperMix, 0.4 μL of each primer (10 μM) and 0.8 μL of cDNA were mixed in 20 μL volumes. The PCR program was achieved with a 2-min activation at 95°C, followed by 40 cycles of 95°C for 10 s, 60 or 58°C for 10 s and 72°C for 20 s. After PCR amplification, melting curve analysis was performed to confirm that only one PCR product was

Table 2 Primer pair sequences and amplicon size of the genes used for real-time PCR

Gene	Nucleotide sequence (5′–3′)	Annealing temperature (°C)	Amplicon size (bp)	Primer efficiency	Accession no.
lpl	F: GAATTCAACGCGGAAACACAG R: ACGCTCATAGAGGGCAGACAC	58	105	0.97	JQ327827
ldlr	F: ACATAAGCGCCGGTGCTGTT R: TACGATGTCCTCTGGCTGATTC	60	95	0.95	KM593127
lrp1	F: TGGACTGGGTGGCTGGAAAC R: CAATGGCGTATGGCTCGTCTATC	60	126	0.95	KM593128
srbi	F: ACAGATCCAGAAAGACAACATCACG R: GTAGGGCAACTTCTCCATCATCAC	60	172	0.95	KM593129
cd36	F: GAGCATGATGGAAAATGGTTCAAAG R: CTCCAGAAACTCCCTTTCACCTTAG	60	159	0.96	KM593122
fatp1	F: CAACCAGCAGGACCCATTACG R: CATCCATCACCAGCACATCACC	60	131	0.98	KM593124
fabp3	F: CCAAACCCACCACTATCATCTCAG R: GCACCATCTTTCCCTCCTCTATTG	60	171	1.01	KM593123
fabp11	F: CAGGTGGGCAATCGGACCAA R: GGCTCGTTGAGCTTGAACTTGA	60	119	0.95	KM593130
fas	F: CAGCCACAGTGAGGTCATCC R: TGAGGACATTGAGCCAGACAC	58	126	0.97	JX456351
dgat2	F: TTCGGTGCTTTCTGCAACTTCG R: AAGGATGGGGAAGCGGAAGT	60	111	0.98	KJ563922
atgl	F: CCATGCATCCGTCCTTCAACC R: GAGATCCCTAACCGCCCACT	60	103	0.98	HQ916211
cptI	F: GCTGAGCCTGGTGAAGATGTTC R: TCCATTTGGTTGAATTGTTTACTGTCC	58	159	0.96	JX434612
aco	F: AGTGCCCAGATGATCTTGAAGC R: CTGCCAGAGGTAACCATTTCCT	58	184	0.97	JX456348
β-*actin*	F: CTACGAGGGTTATGCCCTGCC R: TGAAGGAGTAACCGCGCTCTGT	60	107	0.96	GU584189

lpl, lipoprotein lipase; *ldlr*, low-density lipoprotein receptor; *lrp1*, lipoprotein receptor-related protein 1; *srbi*, scavenger receptor class BI; *cd36*, fatty acid translocase/cluster of differentiation 36; *fatp1*, fatty acid transport protein 1; *fabp*, fatty acid-binding protein; *fas*, fatty acid synthase; *dgat2*, acyl-CoA, diacylglycerol acyltransferase 2; *atgl*, adipose triglyceride lipase; *cptI*, carnitine palmitoyltransferase I; *aco*, acyl-CoA oxidase.

present. Before analysis of the expression of target genes, standard curves for each primer pair were calculated using five differently serial twofold dilution (in triplicate) of cDNA samples, and then, PCR efficiencies were analysed according to the equation: $E = 10^{(-1/\text{slope})} - 1$. After analysis, expression of selected reference gene β-*actin* was stable and independent of dietary lipid levels in muscle and adipose tissue of large yellow croaker, and the amplification efficiencies of the target and reference genes were approximately equal. To calculate gene expression, the comparative CT method ($2^{-\Delta\Delta CT}$ method) was used (Livak & Schmittgen 2001).

Statistical analysis

Data were analysed using a statistical package SPSS version 13 for Windows (SPSS, Chicago, IL, USA). The results were analysed using one-way ANOVA, and Dunnett's test was used to inspect differences between the control group and the treatment groups. If unequal variance was determined using Levene's test, data were log-transformed before statistical analysis. The data were presented as means ± SEM, and the significant level was set at $P < 0.05$.

Results

Moisture and lipid contents in muscle and adipose tissue

The survival rate (81.5–93.9%), final mean weight (269.3–281.6 g) and specific growth rate (0.77–0.85% per day) of the fish were not significantly affected by dietary lipid levels ($P > 0.05$) (Yan *et al.* 2015). Compared with the control group,

muscle lipid content was significantly lower in the low-lipid group, whereas it was significantly higher in the high-lipid group (Table 3; $P < 0.05$). In addition, the lipid content in adipose tissue and moisture content in muscle and adipose tissue were not significantly different in the low- and high-lipid groups compared with the control group (Table 3; $P > 0.05$).

Expression of lipoprotein lipase and lipoprotein receptors

In muscle of fish fed the low-lipid diet, the expression of *lpl* and *lrp1* was significantly downregulated compared with those fed the control diet (Fig. 1a; $P < 0.05$). Compared with the control group, the expression of *lpl*, *ldlr* and *lrp1* in muscle was significantly downregulated in the high-lipid group (Fig. 1a; $P < 0.05$). In adipose tissue of fish fed the high-lipid diet, the expression of *ldlr* was significantly upregulated compared with those fed the control diet (Fig. 1b; $P < 0.05$).

Expression of genes related to fatty acid transport

Compared with the control group, the expression of *fabp3* and *fabp11* in muscle was significantly upregulated in the low-lipid group (Fig. 2a; $P < 0.05$). Compared with the control group, the expression of *fabp11* in muscle was significantly upregulated in the high-lipid group (Fig. 2a; $P < 0.05$). In muscle of fish fed the high-lipid diet, the expression of *fabp3* and *cd36* was significantly downregulated compared with those fed the control diet (Fig. 2a; $P < 0.05$). In adipose tissue of fish fed the low-lipid diet, the expression of *fabp3* was significantly downregulated compared with those fed the control diet (Fig. 2b; $P < 0.05$). In adipose tissue of fish fed the high-lipid diet, the expression of *fabp11* was significantly upregulated compared with those fed the control diet (Fig. 2b; $P < 0.05$).

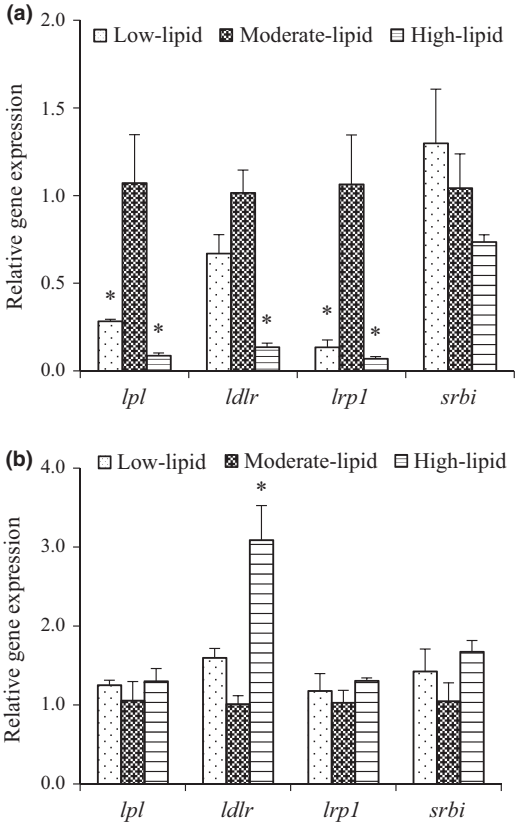

Figure 1 Gene expression of lipoprotein lipase and lipoprotein receptors in muscle (a) and adipose tissue (b) of large yellow croaker fed the experimental diets. Values are expressed as the means ± SEMs ($n = 3$). Statistical significance was evaluated using one-way ANOVA, followed by Dunnett's test. *$P < 0.05$ versus the moderate-lipid group. *lpl,* lipoprotein lipase; *lrp1,* LDL receptor-related protein 1; *srbi,* scavenger receptor class BI.

Table 3 Effects of dietary lipid levels on the moisture and lipid contents of muscle and adipose tissue of large yellow croaker (*Larmichthys crocea*) (%, wet weight)

	Dietary lipid levels (%)		
	Low (6)	**Moderate (12)**	**High (18)**
Muscle (g 100 g^{-1})			
Moisture	74.84 ± 0.24	73.86 ± 0.37	73.39 ± 0.12
Lipid	6.85 ± 0.20*	7.64 ± 0.05	8.39 ± 0.17*
Adipose tissue (g 100 g^{-1})			
Moisture	79.04 ± 0.43	78.44 ± 0.48	79.93 ± 0.39
Lipid	18.82 ± 0.47	19.29 ± 0.41	18.00 ± 0.32

Values are expressed as the means ± SEMs ($n = 3$). Statistical significance was evaluated using one-way ANOVA, followed by Dunnett's test.

*$P < 0.05$ *versus* the moderate-lipid group.

Expression of genes involved in lipogenesis and lipolysis

Compared with the control group, the expression of *fas*, *dgat2* and *atgl* in muscle was significantly

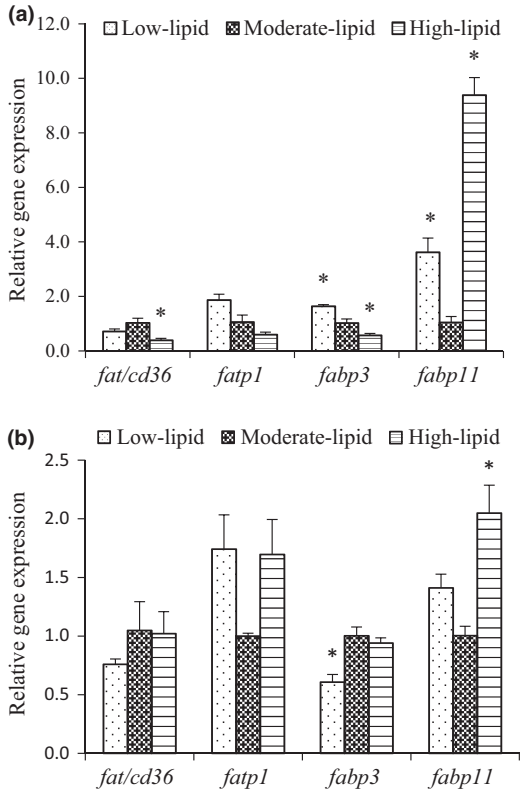

Figure 2 Expression of genes related to fatty acid uptake in muscle (a) and adipose tissue (b) of large yellow croaker fed the experimental diets. Values are expressed as the means ± SEMs (n = 3). Statistical significance was evaluated using one-way ANOVA, followed by Dunnett's test. *P < 0.05 versus the moderate-lipid group. *cd36,* fatty acid translocase/cluster of differentiation; *fatp1,* fatty acid transport protein1; *fabp,* fatty acid binding protein.

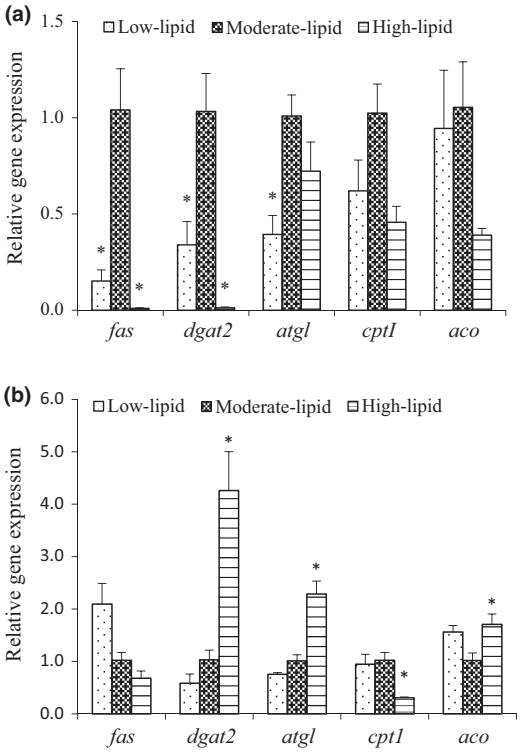

Figure 3 Expression of genes related to lipogenesis and lipolysis in muscle (a) and adipose tissue (b) of large yellow croaker fed the experimental diets. Values are expressed as the means ± SEMs (n = 3). Statistical significance was evaluated using one-way ANOVA, followed by Dunnett's test. *P < 0.05 versus the moderate-lipid group. *fas,* fatty acid synthase; *dgat2,* acyl-CoA:diacylglycerol acyltransferase 2; *atgl,* adipose triglyceride lipase; *cptI,* carnitine palmitoyltransferase I; *aco,* acyl-CoA oxidase.

downregulated in the low-lipid group (Fig. 3a; P < 0.05). Compared with the control group, the expression of *fas* and *dgat2* in muscle was significantly downregulated in the high-lipid group (Fig. 3a; P < 0.05). In adipose tissue of fish fed the high-lipid diet, the expression of *dgat2*, *atgl* and *aco* was significantly upregulated compared with those fed the control diet (Fig. 3b; P < 0.05). In adipose tissue of fish fed the high-lipid diet, the expression of *cpt1* was significantly downregulated compared with those fed the control diet (Fig. 3b; P < 0.05).

Discussion

In the present study, the total lipid content in muscle increased with increasing dietary lipid level, which was in concordance with findings in haddock and Atlantic salmon (Hemre & Sandnes 1999; Nanton *et al.* 2003). However, the total lipid content in adipose tissue was not significantly affected by dietary lipid levels, which was contradictory to the result in rainbow trout that increased lipid content was observed in viscera of fish fed the diet with higher lipid (Jobling *et al.* 1998). These inconsistent results may be related to the fact that the effect of dietary lipid level on lipid metabolism in fish adipose tissue is usually species specific (Weil, Lefèvre & Bugeon 2013).

LPL serves as a 'gatekeeper' to direct calories/lipids to specific tissues (Goldberg *et al.* 2009). In large yellow croaker, *lpl* is highly expressed in tissues like adipose and muscle that require large

influxes of fatty acids for storage or energy (Cai, Xie, Mai & Ai 2015). LRP1, as a chylomicron remnant receptor and in a synergistic action with LPL, also mediates the uptake of lipid from remnant lipoproteins (Descamps *et al.* 1993). In the present study, the gene expression of *lpl* and *lrp1* was downregulated in muscle of fish fed the low-lipid diet, which probably indicated a reduction in lipid uptake in muscle of fish fed the low-lipid diet. FAS is a key enzyme involved in *de novo* lipogenesis, and DGAT catalyses the final and only committed step in the biosynthesis of TAG (Coleman & Lee 2004). ATGL has been proven to catalyse the initial step of TAG hydrolysis and that *atgl* mRNA level in the liver of large yellow croaker was regulated by dietary lipid source and level (Wang, Wang & Li 2013). In the present study, the gene expression of *fas*, *dgat2* and *atgl* in muscle was also downregulated by low-lipid diet. It may be that the decrease in lipid uptake reduces fatty acid-CoA, a necessary intermediate for TAG synthesis, and further inhibits TAG synthesis and hydrolysis in muscle of fish fed the low-lipid diet. Additionally, in the present study, the gene expression of *fabp3* and *fabp11* in muscle was upregulated by low-lipid diet. The result was inconsistent with previous findings that *fabp3* mRNA level in the liver of zebrafish was downregulated by low-lipid diet (Venkatachalam *et al.* 2013), but consistent with findings that *fabp11a* mRNA level in muscle of zebrafish was upregulated by low-lipid diet (Karanth *et al.* 2009). Previous studies have indicated that *fabp3* mRNA expression correlated to β-oxidation in Atlantic salmon red muscle (Jordal *et al.* 2006) and white muscle (Torstensen *et al.* 2009). Furthermore, FABP11, the orthologue to the mammalian adipocyte FABP4 (Karanth, Denovan-Wright, Thisse, Thisse & Wright 2008), plays a role in lipid storage in the adipose tissue (Storch & Thumser 2010), and a similar role in muscle of fish was also reported that the expression of *fabp11* was correlated with the lipid content in Atlantic salmon white and red muscle (Torstensen *et al.* 2009). In the present study, however, *fabp11* expression in muscle of fish fed the low-lipid diet is not directly related to lipid storage. The increased expression of *fabp11* in muscle of fish fed the low-lipid diet may be associated with other lipid metabolism pathways.

In muscle of fish fed a high-lipid diet, the gene expression of *fabp11* was upregulated, whereas *fabp3* was downregulated. Increased *fabp11* mRNA level with increased lipid content supported a role of FABPP11 in lipid storage. Meanwhile, although β-oxidation-related gene (*cpt1* and *aco*) was not significantly decreased, the reduced *fabp3* expression probably suggested the decreased capacity for β-oxidation in muscle of fish fed a high-lipid diet, because high-lipid diet induced a decrease in β-oxidation in the liver of large yellow croaker (Yan *et al.* 2015) and in muscle of mammals (Begriche, Massart, Robin, Bonnet & Fromenty 2013). In addition to *fabp3*, the lipid uptake-related genes, including *lpl*, lipoprotein receptors (*ldl* and *lrp1*) and fatty acid transport protein *cd36*, were downregulated by high-lipid diet in muscle of large yellow croaker. One possible explanation for the decreased expression of these genes is a self-regulation mechanism, in which the downregulation of lipid uptake-related genes serves a protective function against lipotoxicity and excess lipid deposition through inhibition of lipid uptake (Van Herpen & Schrauwen-Hinderling 2008). Additionally, high-lipid diet inhibits *fas* expression to decrease *de novo* lipogenesis in the liver of large yellow croaker (Wang *et al.* 2015). Accordingly, high-lipid diet inhibited *fas* as well as *dgat2* expression in muscle of large yellow croaker in the present study. The downregulation of *fas* and *dgat2* in muscle of large yellow croaker might be driven by the increased lipid accumulation through a feedback mechanism. Alternatively, the inhibitory role of lipogenesis by increased n-3 long-chain polyunsaturated fatty acid level in the high-lipid diet could be another factor related to the reduction in *fas* and *dgat2* expression (Alvarez, Diez, Lopez-Bote, Gallego & Bautista 2000; Kjaer, Vegusdal, Gjøen, Rustan, Todorčević & Ruyter 2008).

In adipose tissue, the expression of all genes we measured (except for *fabp3*) had no significant changes in response to low-lipid diet, suggesting a little effect of low-lipid diet on lipid metabolism in adipose tissue of large yellow croaker. The expression of *fabp3*, as an adipogenic gene marker, increased during Atlantic salmon preadipocytes differentiation (Todorčević *et al.* 2008). Therefore, the decrease in *fabp3* expression in adipose tissue of fish fed the low-lipid diet probably indicates a reduced preadipocytes differentiation.

Adipose tissue plays an important role in storing excess dietary lipid (Greenberg & Obin 2006). When energy intake exceeds energy expenditure the energy surplus causes the differentiation of

preadipocytes and enhanced lipid uptake and TAG synthesis, resulting in enhanced adipogenesis (Greenberg & Obin 2006). Moreover, during adipogenesis, *fabp11* expression was elevated in mature salmon adipocytes (Huang, Todorčević, Ruyter & Torstensen 2010). In the present study, the lipid uptake-related gene *ldlr*, the TAG synthesis-related gene *dgat2*, and *fabp11* mRNA levels all increased in adipose tissue of fish fed the high-lipid diet, probably indicating an enhanced adipogenesis in fish fed the high-lipid diet. In addition, triacylglycerol in adipose tissue can also be hydrolysed to diacylglycerol *via* the ATGL enzyme and eventually to non-esterified fatty acids (Arner & Langin 2014). In mammals, the upregulated ATGL with enhanced lipolysis in adipocytes is the main contributor to increased plasma free fatty acid and lipid deposition in the liver (Arner & Langin 2014). In a study on gilthead sea bream, the authors also suggested that an increase in the adipocyte lipolytic rate in adipose tissue could produce an increase in lipid accumulation in the liver (Cruz-Garcia, Sánchez-Gurmaches, Bouraoui, Saera-Vila, Pérez-Sánchez, Gutiérrez & Navarro 2011), as already proposed by Benedito-Palos, Navarro, Sitjà-Bobadilla, Bell, Kaushik and Pérez-Sánchez (2008) and Ibarz, Blasco, Gallardo and Fernandez-Borras (2010) in gilthead sea bream. Therefore, the upregulation of *atgl* in fish fed the high-lipid diet potentially suggested a higher lipolysis rate of adipose tissue, which could affect lipid deposition in the other tissues of large yellow croaker.

In conclusion, this study describes for the first time the effects of dietary lipid levels on lipid metabolism, involving lipid uptake, lipogenesis and lipolysis at the transcriptional level in muscle and adipose tissue of large yellow croaker. Results of the present study indicated that the expression of the selected key genes related to lipid metabolism was tissue-specific regulation by dietary lipid levels in large yellow croaker.

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